Use of scanning electron microscopy to confirm the identity of tropical rat mite (*Ornithonyssus bacoti*): the cause of rat mite dermatitis

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Abstract Cutaneous lesions in human patient due to the bite of rat mite *Ornithonyssus bacoti* are frequently misdiagnosed as allergies, fungal infection, or bacterial infection. Bite lesions in the personnel working in a Laboratory Animal facility which was infested with *O. bacoti* is reported here along with its therapeutic management. Diagnosis of the parasites obtained from the clothing of the personnel and later from the infested mice colony was based on preliminary light microscopy and confirmed by scanning electron microscopy. The mean length and breadth of adult female mite were 1.13 mm × 0.63 mm. The body is hairy, unsegmented and has four pairs of legs. The gnathostoma has long pointed chelicerae and pedipalp. The dorsal surface has one dorsal shield, and the ventral surface has three shields- sternal, genital and anal shield. Treatment of dermatitis involved antihistaminic drugs for a period of 3–5 days. The skin lesion, characterized by papular erythema, tends to disappear within a period of 4–5 days of antihistaminic treatment. In untreated cases, the lesions disappeared within 7–10 days. Tropical rat mite *O. bacoti* Hirst, 1931 was identified to be the cause of infestation in the laboratory mice colony of Pasteur Institute of India, Coonoor, Tamil Nadu, predisposing the animal handlers to be temporary host.

Keywords *Ornithonyssus bacoti* · Rat mite dermatitis · Scanning electron microscopy · Tropical rat mite

Introduction

Different species of mite occurring in animals may temporarily infest human. Mesostigmatid mites are a large order of highly mobile mites, Class Arachnida, Subclass Acari, Order Mesostigmata (Watson 2008). Wild rodents are host to different mite species including *Ornithonyssus bacoti*. *O. bacoti* has the widest non selective host range including man and laboratory rodents (Watson 2008). This mesostigmatid mite is a rapid blood sucker and spends much of their life cycle in the surrounding environment except during feeding on the host. Tropical rat mite is reported to be present worldwide. In India, there is limited information available about the distribution of tropical rat mite *O. bacoti* in rodents as well as human cases of tropical rat mite dermatitis. This may be due to non-reporting of such cases and failure to detect or diagnose the parasite. The definitive diagnosis of this ectoparasitosis requires the detection of the parasite, which is more likely to be found in the environment of its host (in the cages, in the litter or in corners or cracks of the living area) than on the hosts' skin itself (Beck 2008). In India, the first report of *O. bacoti* infestation in laboratory personnel and veterinary student was reported by Tika-Ram et al. (1986) from the state of Haryana. In a recent publication by Nath et al. (2013), the preliminary report of this incidence covering the diagnosis based on light microscopy and treatment and control aspect in the infested laboratory mice colony was reported from the temperate hilly district of Tamil Nadu. From zoonotic point of view, *Bartonella* species, *Coxiella burnetii*, and *Rickettsia* species have been reported to be detected in *O. bacoti* (Reeves et al. 2007). Hence occurrence of such parasitic infestations has to be considered serious and adequate control measures to be adopted. Light microscopy does not reveal detail morphology to classify the parasite into their taxonomic level. The

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main aim of this investigation was to study the micro-morphological features of *O. bacoti* using scanning electron microscopy which could aid in the identification and differentiation from other closely related parasites and its treatment aspect in animal handlers exposed to such infestation.

Materials and methods

Case report

The laboratory animal facility of the Pasteur Institute of India houses random bred conventional colonies of Swiss mice and guinea pigs with proper physical separation between the two species. The animals are reared as per the guidelines of Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA). The infestation of the mice colony reported here was brought to our notice by the personnel working in the infested colony as they complained of itching and non-specific lesions in different parts of their body, mostly on the day during cage changing and cleaning operations. On physical examination of the individuals papular lesions (approximately 3–5 mm in diameter, surrounded by mild erythematous halo) were observed in different parts of the body, mostly on upper and lower limbs, sometimes on back, scapular region and over the shoulder. Figure 1 shows the bite lesions in the affected personnel. The lesions in the lower part of the body were restricted till the waist. All the individuals working in the infested mice colony were found to be affected. Personnel working in the laboratory animal facility are provided with the requirements for maintaining proper hygiene and cleanliness viz wash basin, toilet, bathroom etc. along with protective clothing. A routine health monitoring practice is in place for both the personnel working in the facility and the animals housed.

On careful examination mites were observed on the clothing of personnel, and also on the cages of mice. Thus the lesions found on laboratory personnel can be attributed to the bite of mites. Further it is assumed that the natural constriction of the trousers over the waist offers a barrier to the movement of the mite, restricting the lesions in the lower part of the body.

Treatment

In untreated cases, the lesions subsided within 7–10 days. In some patients oral administration of anti-histaminic drugs viz. CETRAL O (Cetirizine Hydrochloride 10 mg, Acme Formulations Pvt. Ltd, Nalagarh, Himachal Praesh, India) or Avil 25 (Pheniramine Maleate 25 mg, Sanofi India Ltd, Ankleshwar, India), once daily for a period of 3–5 days could reduce the severity of itching as well as the duration of the lesions to 4–5 days. No difference could be observed in the outcome of using CETRAL O or Avil.

Collection of mites

The unfed mite was observed as tiny white/grey dots moving actively in the personnel clothing and in the animal cages, while the blood engorged mite was observed as sluggishly motile and red/brown in colour. The individual mite samples were collected in 10 % formaldehyde solution and sent to the laboratory for detail study.

Laboratory examination

The mite samples after overnight treatment with lactophenol were mounted in glass slides and studied under a compound light microscope in 10 ×, 40 × and 100 × objectives using 10 × eyepiece. This preliminary

Fig. 1 Bite lesions in the animal handlers over the chest (a) and in hand (b)

study was further supported by micro-morphological studies using scanning electron microscopy.

For scanning electron microscopy the samples were washed several times with distilled water to remove the dirt and other extra materials over the surface. They were fixed in 3 % glutaraldehyde at 4 °C for 4 h and transferred into 0.2 M cacodylate buffer at 4 °C overnight, dehydrated in ascending grades of acetone and dry acetone followed by drying in teramethyl silane (TMS) (Dey 1993). They were coated with gold in an ion sputter (JEOL, Fine Coat, Japan) and examined under a scanning electron microscope (JEOL, JSM-6360, Japan) at an accelerating voltage of 20 kV to record the surface structure of the parasites.

Results and discussions

The light microscopy images are presented in Fig. 2. The body is unsegmented, elongated oval in shape. Adult parasites have four pairs of legs. The mean length and breadth of the female parasites were 1.13 mm × 0.63 mm. Scanning electron microscopy revealed that the dorsal shield is narrow and tapering towards the posterior side ending in a blunt point (Fig. 3a). There are 11 pairs of pilose setae on the dorsal shield. The length of the setae on dorsal shield was same to the setae on the surrounding area. The ventral surface of female mites have three shields, sternal shield, genital shield and anal shield (Fig. 3b). The gnathosoma has chelicera and pedipalp (Fig. 4a). The chelicera is long and pointed which is very clearly visible in light microscopy, cleared during processing with lactophenol (Fig. 2). Each trochanter of pedipalp has an anteroventral keel. Tritosternum is long and serrated (Fig. 4a). Sternal shield

has three pairs of pilose setae (Fig. 4b) of which the anterior pair is nearer the anterior margin of the shield. Genital shield has a pair of genital setae (Fig. 4b). Anal shield has three setae. The anus is located in the anterior half of the anal shield. Posterior part of the anal shield has a large number of spines arranged in V-shape extending just anterior to the terminal anal seta (Fig. 4c). The above micro-morphological characteristics as observed by scanning electron microscopy is as per the descriptions of other published literature for *O. bacoti* Hirst, 1931 (Watson 2008; Soulsby 1982; Engel et al. 1998). Certain morphological characteristics viz. hairiness, caudally pointed dorsal plate, typical form of anal plate with a cranial anus allow for differentiation of the tropical rat mite from other closely related parasites like red bird mite (*Dermanyssus gallinae*) and Nordic bird mite (*Ornithonyssus sylvarium*) (Beck and Fölster-Holst 2009). There is scanty report on the microscopy of developmental stages of the parasite. However, the review on this parasite's life cycle by Engel et al. (1998) provide information to differentiate all the developmental stages including eggs, larva, protonymph, deutonymph and adult based on their morphological characters viz. size and number of legs. Scanning electron microscopy creates a three dimensional image of the surface structure of an object. Because of its higher resolution capacity up to 2 million times, as compared to 1,000–2,000 times in light microscopy, electron microscopy allow for study of micromorphological structures not visible by light microscopy. However, electron microscopy is expensive and need expertise.

Several species of mite including *Sarcoptes scabiei*, *Notoedres cati*, *Cheyletiella* spp., *D. gallinae*, *O. bacoti*, *Ophionyssus natricis* and *Neotrombicula autumnalis* may

Fig. 2 Light Microscopy image of ventral surface of *O. bacoti*, 40 × (a) and the gnathosoma showing two pedipalps and long chelicerae, 100 × (b)

Fig. 3 SEM image of dorsal surface of *O. bacoti* showing the dorsal shield and setae (a) and the ventral surface showing sternal, genital and anal shield (b)

infest human skin, causing symptoms (Beck and Pfister 2006). Mites may produce pruritis or allergic reaction through salivary proteins deposited during feeding (Scharf and Daly 2003). The common hypersensitivity reaction to all arthropod bites, stings and products is referred to as papular urticaria (Steen et al. 2004). Mite species that occur in animals may be the cause of temporary human infestation. One of such temporary infestation in human occurs in case of *O. bacoti*, otherwise known as Tropical rat mite. *O. bacoti* is periodic hematophagous parasite and spend relatively short time on a host. When the rat is trapped, or it has abandoned its living space, the mite may cause infestation in search of food. Tropical rat mite has been reported to occur in many countries except the arctic and antarctic region (Engel et al. 1998).

Casual therapy by antiparasitic agents in human patients is not necessary, however, if indicated, symptomatic treatment can be provided (Beck 2007). Different authors

Fig. 4 SEM image of gnathosoma of *O. bacoti* showing the chelicera and pedipalp (a), sternal shield showing 3 pairs of pilose setae (b) and the anal shield showing 3 anal setae and V-shaped arrangement of spines (c)

have reported use of antihistaminic drug (Hetherington et al. 1971), glucocorticoids (Fishman 1988), lindane (Fishman 1988), and 25 % benzyl benzoate (Tika Ram et al. 1986). In our case, we used antihistaminic treatment alone, only in few patients, as the severity of the lesions we

encountered was very less. Some of the patients experienced severe itching, hence antihistaminic drug was prescribed. The itching subsided following antihistaminic drug and the lesions tend to disappear in shorter period in patients administered antihistaminic drugs. The recovery was uneventful within a period of 5–10 days.

In laboratory animal facility a comprehensive eradication programme should ideally include elimination of commensal rodent reservoir, treating the infested animals and their environment. However, reinfestation cannot be ignored if the rat control programme fails. Building demolition and renovations or removal of commensal rodents are particularly high-risk periods and can result in new laboratory rodent mite infestations as the mites travel in search of new hosts (Watson 2008). It has also been reported that the mite can survive 2 weeks to several months without feeding (Baker 1998). The control programme in our facility included eradication of commensal rodent reservoir in the Laboratory Animal Facility, the use of readily available deltamethrin (Butox vet[®]), a pyrethroid group insecticide, to treat the infested colony and its environment (Nath et al. 2013). Other pyrethroid group of drugs, viz. permethrin, a synthetic pyrethroid (Hill et al. 2005, Cole et al. 2005), either alone or in combination with sustained released deltamethrin (Watson 2008) have been used successfully to treat the infestation in a laboratory mice colony.

This study concluded that the cases of dermatitis in the personnel working in the Laboratory animal Facility was due to the bites of *O. bacoti* which originated from infested mice colony. Scanning electron microscopy was useful to confirm the identification of the parasite based on the studies of micromorphological structures. In India, there is limited information on the occurrence of *O. bacoti* infestation and dermatitis caused by *O. bacoti*. Although no recorded cases are available, the potential of this mite in transmitting disease need to be viewed seriously. Symptomatic treatment, as and when required, may be helpful to reduce the severity of itching.

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